MINIREVIEW

Aspergillus biofilms: clinical and industrial significance

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Abstract

The biofilm phenotype is an increasingly important concept in mycological research. Recently, there has been a developing interest in whether *Aspergillus* species are truly able to form biofilms or not. Industrial mycologists have long been aware of biofilms and their benefit in fermentation processes, whereas clinically their role is uncertain. This review provides an update on the impact that *Aspergillus* biofilms have medically and industrially, and will discuss biofilm development, and our current understanding of its molecular basis. The role of exopolymeric substance and how this substance relates to antimicrobial recalcitrance will also be discussed.

Introduction

Mycological research has observed a paradigm shift in recent years, with a developing appreciation that fungi of clinical importance have the capacity to survive within the host comprised of biofilm communities (Jabra-Rizk *et al.*, 2004; Ramage *et al.*, 2009; Martinez & Fries, 2010). This is particularly true for *Candida albicans*, where its ability to form biofilms upon biomaterials such as catheters and dentures, or residing upon mucosal surfaces, has been fully realized (Ramage *et al.*, 2006). A consequence of this has been an extensive research effort resulting in an improved understanding of the physiology, biochemistry and molecular cell biology of these structures (Finkel & Mitchell, 2011). This has enabled researchers to learn more about the complex molecular pathways that govern biofilm development, and from a translational standpoint devise new and improved strategies to control these hard-to-treat infections (Nett *et al.*, 2010b). Given the complex intertwined growth characteristics that *Aspergillus fumigatus* exhibits *in vivo*, there has recently been a growing body of literature to support the idea that it has the capacity to exist as biofilm (Beauvais *et al.*, 2007; Mowat *et al.*, 2008a; Bruns *et al.*, 2010; Gravelat *et al.*, 2010; Loussert *et al.*, 2010; Muller *et al.*, 2011; Singhal *et al.*, 2011). This review will present the latest evidence to support the evolving concept, that clinically, *Aspergillus* species can form biofilms.

Biofilms, what is in a definition?

There has been much debate within the mycology community of what specifically constitutes a biofilm. The ability of fungi to attach to a surface and/or to one another, and to be enclosed within an exopolymeric substance (EPS) is sufficient to fit the basic criteria of a microbial biofilm. From the available literature, it is increasingly clear that different *Aspergillus* species do have this overall capacity, which is hardly surprising given that 80% of all microorganisms are proposed to exist within multicellular communities. Moreover, 65% of human infection is biofilm associated, which is related to increasing number of immunocompromised patients and the escalating use of biomaterials in medicine (Donlan, 2002; Lopez-Ribot, 2005; Ramage *et al.*, 2005; Blankenship & Mitchell, 2006). Moreover, review of the literature highlights that industrial mycologists have been aware of the beneficial aspects of *Aspergillus* biofilms for some time

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(Villena & Gutierrez-Correa, 2007b). Therefore, it is clear that *Aspergillus* species have developed ways of coordinating their behaviour to form biofilms, which impact clinical medicine and industrial processes.

Clinical and industrial significance

Aspergillosis is a debilitating disease affecting an expanding population of immunocompromised patients, and is associated with high rates of mortality. Patients who are particularly susceptible include those who are neutropenic following chemotherapy, transplant, surgical and ICU patients (Ben-Ami *et al.*, 2009; Zilberberg & Shorr, 2009). Moreover, patients with genetic or functional abnormalities, particularly in the lungs such as those with cystic fibrosis (CF) or chronic obstructive pulmonary disease provide a natural environment that has a predilection for *Aspergillus* colonization and biofilm formation (Bakare *et al.*, 2003; Ader *et al.*, 2009; Horre *et al.*, 2010; Moss, 2010).

Aspergillus produce small spores called conidia that have an average size of 2–3.5 μm. These are dispersed in the air and remain in the atmosphere for prolonged periods, and are inhaled into the respiratory tract in their hundreds each day by humans and other mammals (Rivera *et al.*, 2006). *Aspergillus fumigatus* can cause a spectrum of clinical disease, including allergic bronchopulmonary aspergillosis, an aspergilloma or invasive aspergillosis (IA) (Denning, 1998). Of these the aspergilloma, a localized infection consisting of a spherical mass of hyphae has clear biofilm characteristics. Aspergillomas can develop in immune competent hosts, but usually require a pre-existing cavity such as those resulting from prior tuberculosis. Some are asymptomatic; however, where symptoms exist, they commonly include a chronic cough and haemoptysis. Another form of aspergillosis infection, aspergillary bronchitis, is characterized by bronchial casts containing mucus and mycelia, which are associated with pathological damage (Young *et al.*, 1970). Compact masses are formed, which may be expectorated. Moreover, bronchoalveolar lavage (BAL) in some patients with aspergillosis reveals the presence of numerous hyphae in the form of a complex multicellular mycetoma structure samples when examined histologically (Jayshree *et al.*, 2006). In contrast, IA disease is more diffuse with multiple points of angioinvasion within the pulmonary tissue. Nevertheless, filamentous intertwined hyphae are important to this process, as in other forms of aspergillosis (Mowat *et al.*, 2007). Notably, antifungal treatment is often ineffectual, which may relate to the biofilm phenotype (Beauvais *et al.*, 2007; Mowat *et al.*, 2007, 2008b; Seidler *et al.*, 2008; Fiori *et al.*, 2011; Rajendran *et al.*, 2011).

Clearer evidence of *Aspergillus* biofilms is demonstrated in infections affecting other sites. Aspergilli can enter the host through alternative routes causing other serious biomaterial-related biofilm infections, including catheters, joint replacements, cardiac pace makers, heart valves and breast augmentation implants (Rosenblatt & Pollock, 1997; Langer *et al.*, 2003; Escande *et al.*, 2011; Jeloka *et al.*, 2011). *Aspergillus* is also frequently associated with complex sinus infections, which in canines have been described as superficial mucosal fungal plaque (Grosjean & Weber, 2007; Day, 2009; Laury & Delgaudio, 2010; Sato *et al.*, 2010). The urinary tract, whilst less frequently associated with *A. fumigatus*, has been reported to support an aspergilloma (Lee, 2010; Muller *et al.*, 2011). One such recent case study described an *Aspergillus flavus* aspergilloma in a neonate who had urinary catheters placed for genitourinary complications (Martinez-Pajares *et al.*, 2010).

Aspergillus species of industrial importance can also be problematic. For example, adhesion of *Aspergillus niger* spores may cause surface deterioration on different substrates, and has also been associated with colonization of contact lenses (Marques-Calvo, 2002). However, many of the characteristics associated *Aspergillus* biofilms are beneficial with respect to industrial processes. Various organic acids have been produced by *Aspergillus* biofilms using different supports and bioreactors. In one of the oldest publications, *A. niger* was grown attached to the vertical discs of a rotating disc reactor (Blain *et al.*, 1979), producing fourfold higher citric acid titres than in stirred tank reactor (Anderson *et al.*, 1980). It was also found that *A. niger* immobilized on polyurethane foam (biofilms) in a bubble reactor for citric acid production performed better than free-living pellets (Lee *et al.*, 1989). Other organic acids have been produced by *Aspergillus* biofilms. For example, *Aspergillus terreus* grown attached on polyurethane foam used for itaconic acid production (Kautola *et al.*, 1989), gluconic acid has also been produced by passively immobilized *A. niger* (Vassilev *et al.*, 1993; Fiedurek, 2001). Moreover, several enzymes have been produced by *Aspergillus* biofilm systems, such as the production of glucose oxidase, inulinase, amylase and cellulases by *A. niger* (Fiedurek & Ilczuk, 1991; Murado *et al.*, 1994; Skowronek & Fiedurek, 2006; Gamarra *et al.*, 2010), production of β-frutofuranosidase by *Aspergillus japonicas* (Mussatto *et al.*, 2009) and production of xylanases by *A. terreus* and *A. niger* (Gawande & Kamat, 2000). *Aspergillus foetidus* biofilms have been shown to degrade some plastics under growth (Upreti & Srivastava, 2003). Also, *Aspergillus versicolor* has been found to form biofilms on perlite particles in a packed column reactor, and in this condition, it could degrade *n*-alkanes, aromatic hydrocarbons and carbazoles of petroleum samples (Sanchez *et al.*, 2006). Removal of heavy metals (copper, chromium, iron and nickel) by biosorption of either

A. niger or *A. terreus* biofilms formed on polyurethane, has also been reported to be a highly efficient method of metal removal (Tsekova & Ilieva, 2001; Dias *et al.*, 2002). Clearly, *Aspergillus* biofilms are important in many industrial processes, particularly because they are much more productive than in the classical submerged fermentation with free-living mycelia.

Adhesion

Filamentous growth is a fundamental feature of fungal biofilms and is an important morphological characteristic of *A. fumigatus* required during the development of an aspergilloma (Beauvais *et al.*, 2007; Ramage *et al.*, 2009; Loussert *et al.*, 2010). The initial establishment of these chronic infections involves the germination of conidia, and subsequent hyphal invasion of the lung tissues (Filler & Sheppard, 2006). Fungal spores adhere to compatible surfaces through several mechanisms, which include complex interactions of physical and biological processes. Physical properties of support like hydrophobicity, electrostatic charge and surface roughness are important at the initial adhesion step of bacteria, as well as yeasts and filamentous fungi (Cunliffe *et al.*, 1999; Webb *et al.*, 1999; Dufrene, 2000; Bigerelle *et al.*, 2002; Beauvais *et al.*, 2007).

A small class of amphipathic proteins called hydrophobins principally mediate adhesion in filamentous fungi, and have recently been shown to play a role in fungal biofilm development (Kershaw & Talbot, 1998; Linder *et al.*, 2005a; Armenante *et al.*, 2010; Bruns *et al.*, 2010; Perez *et al.*, 2011). Hydrophobins stabilize the adhesion of spores to both natural and artificial hydrophobic surfaces, possibly generating morphogenetic signals (Scholtmeijer *et al.*, 2001; Wosten, 2001; Linder *et al.*, 2005b). Hydrophobins, a family of small-secreted proteins with a characteristic pattern of eight cysteine residues, have been reported in *A. fumigatus* to be responsible for the strong adhesion forces of 2858 ± 1010 pN during spore adhesion to surfaces (Dague *et al.*, 2008; Dupres *et al.*, 2010). It seems that conidium contact/attachment is required to trigger germination (Shaw *et al.*, 2006).

It has been shown that when *A. niger* biofilms are under stress caused by low water activity (a_w), high amounts of exopolymeric material are secreted (Villena & Gutierrez-Correa, 2007a). In some plant pathogenic fungi like *Bipolaris sorokiniana*, the production of EPS appears to be important for adhering conidia and germlings to the host surface (Apoga *et al.*, 2001). For the development of *A. niger* biofilms, the spore rough surface is important for its first physical attachment to the support surface and this process is also helped by the production of adhesive substances forming a pad beneath spores; this has been found when different supports were used, indicating that the adhesive substances are part of the adsorption process (Villena & Gutierrez-Correa, 2007b; Gamarra *et al.*, 2010; Lord & Read, 2011).

Further studies of the genetic basis of biofilm formation has revealed a role for *medA*, which has recently been characterized with respect to conidiation, host cell interactions and virulence (Gravelat *et al.*, 2010). Herein, it was reported that in addition to its role in conidiophore morphology, it was shown that its mutant phenotype was impaired in biofilm production, in addition to adherence to plastic, pulmonary epithelial cells, endothelial cells and fibronectin *in vitro*. Moreover, this strain had reduced capacity to damage pulmonary epithelial cells and stimulate pro-inflammatory cytokines, which was reflected by reduced virulence in both an invertebrate (*Galleria mellonella*) and a mammalian model (mouse) of IA (Gravelat *et al.*, 2010).

It was also shown that deletion of *cspA* (cell surface protein A), which encodes a repeat-rich glycophosphatidylinositol-anchored cell wall protein, causes weakening of the conidial cell wall (Levdansky *et al.*, 2010). Analysis of double mutants indicated that *cspA* interacts with the cell wall protein-encoding genes *EPS33* and *Gel2*, deletion of which results in strongly reduced conidial adhesion, increased disorganization of the conidial cell wall and exposure of the underlying layers of chitin and beta-glucan. Given the number of genes integral to adhesion and cell wall structure, it is likely that many play a pivotal role in the different phases of biofilm formation, and as biofilm becomes an accepted term within the *Aspergillus* research community, many future studies will elucidate the molecular pathways for its development.

Biofilm development

The initial clinical-based studies explored whether *A. fumigatus* multicellular structure (or mycelial mass) fits the criteria for a biofilm, and represents a source of continuing debate (Chandrasekar & Manavathu, 2008; Mowat *et al.*, 2008a). A key factor as observed early in our investigations was the critical importance of conidial seeding density, a phenomenon also described in studies of *A. niger* biofilms (Villena & Gutierrez-Correa, 2006). Confocal laser scanning microscopy (CLSM) and scanning electron microscopy (SEM) demonstrated an optimal conidial seeding density of 1×10^5 conidia mL^{-1} of liquid medium. Therefore, structural morphology and integrity was dependent upon the concentration of conidia (Mowat *et al.*, 2007). This biofilm system was then amenable to high throughput testing required for the screening of clinical isolates and defined mutants, or for testing the susceptibility of antifungal agents (Mowat

et al., 2008b). This concentration differs from those described elsewhere, where it was reported than an optimized conidial density of 1×10^6 per millilitre was used, a concentration also reported within an epithelial-biofilm co-culture system (Villena & Gutierrez-Correa, 2006; Seidler *et al.*, 2008). However, this log difference was due to subtleties of biofilm model systems, which both utilize different substrates and media.

Fungal biofilms, like bacterial biofilms, have defined developmental phases that include arrival at an appropriate substrate, adhesion, colonization, polysaccharide production, and biofilm maturation and dispersal (Chandra *et al.*, 2001; Donlan, 2002; Blankenship & Mitchell, 2006). Both CLSM and SEM were used in our investigations to evaluate the characteristics of this development. Following initial conidial seeding, there is a lag phase (conidial adhesion), germination (6–8 h), filamentation and formation of a monolayer (12 h), followed by increased structural complexity, EPS production and maturation (24 h). During this time, the depth of the biofilm increases from 10 to 200 μm (Mowat *et al.*, 2007). These phase dependant growth characteristics play a key role in the outcome of antifungal treatment, of which efflux- and EPS-mediated resistance are both implicated (Rajendran *et al.*, 2011). The development of *A. fumigatus* biofilms is illustrated in Fig. 1.

The process of *A. niger* biofilm formation can also be divided into distinct phases: (i) adhesion, which is strongly increased by *A. niger* spore hydrophobicity, (ii) an initial growth and development phase from spore germination to surface colonization and (iii) a maturation phase, in which biomass density is highly increased with development of an internal channel organization (Gutierrez-Correa & Villena, 2003). These channels appear to allow fluids to pass through, favouring a better mass transfer (Villena & Gutierrez-Correa, 2006; Villena & Gutierrez-Correa, 2007b; Villena *et al.*, 2010). There is also different spatial growth coordination when fungus adheres to the surface. This coordination responds to steric interactions between hypha and tips in contact with surfaces. At short distances, binary interactions (tip–hyphae) involve a local spatial rearrangement, resulting in a slowing down of the tip extension rate and consequently in a control of maximum biomass surface density (Villena *et al.*, 2010).

Molecular mechanisms

Very few reports on the molecular biology and functional genomics of *Aspergillus* biofilms have been published; however, a recent study reported global transcriptional and proteomic biofilm specific changes in *A. fumigatus* (Bruns *et al.*, 2010). Planktonic- and biofilm-grown mycelium at 24 and 48 h growth was analysed using microarrays and 2D gel electrophoresis. Both biofilm- and time-dependent regulation of many proteins and genes associated with primary metabolism was demonstrated, indicating an energy-dependant developmental stage of young biofilms. Biofilm maturation showed a reduction of metabolic activity and an upregulation of hydrophobins, and proteins involved in the biosynthesis of secondary metabolites, such as gliotoxin (Bruns *et al.*,

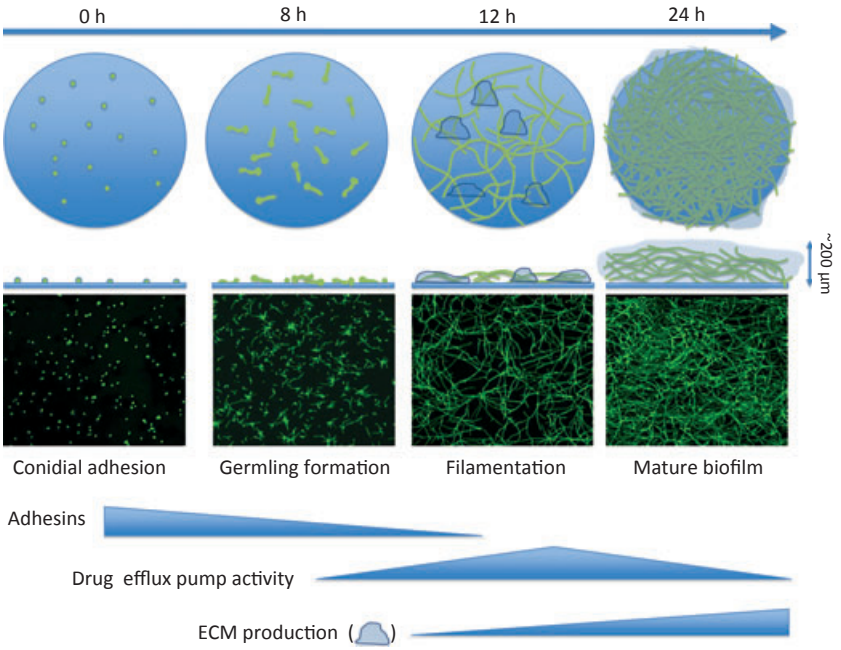

Fig. 1. *Aspergillus fumigatus* biofilm development. The different phases of biofilm development are represented schematically, from initial adhesion of conidia, germling formation (8 h), a monolayer of intertwined hyphae (12 h) and mature 3D filamentous biomass (c. 200 μm) encased within EPS (24 h). These are also illustrated in the adjacent confocal laser scanning micrographs stained using FUN1 (Molecular Probes). Key classes of genes and their optimal time of expression are indicated.

2010). Specifically, it was shown that 36 protein spots changed in biofilm mycelium of *A. fumigatus* in comparison to planktonic mycelium, and 78 protein spots changed significantly during biofilm maturation. Based on FunCat categorization these included – proteins involved in 'metabolism', 'protein with binding function or cofactor requirement' and 'cellular transport, transport facilitation and transport routes'. Transcriptional profiling demonstrated that 740 genes were differentially regulated (179 up- and 561 down-regulated) with respect to 24 h biofilm vs. planktonic cells. The up-regulated genes were mainly involved in protein synthesis, metabolism, energy conservation and encoded for proteins with binding function or cofactor requirement. Many down-regulated genes were involved in signal transduction, cell type differentiation, interaction with the environment, biogenesis of cellular components, regulation of metabolism and protein function, as well as cell and protein fate (Bruns *et al.*, 2010). Recent work has used RNA-Seq to compare the transcriptomes of biofilm and liquid planktonic growth, where sequencing identified 3728 differentially regulated genes in the two conditions (Gibbons *et al.*, 2011). In addition to many genes that are likely to reflect the different growth demands, these investigations identified many up-regulated genes involved in transport, secondary metabolism and cell wall and surface functions. Mapping of these genes showed significant spatial structure across the genome. A total of 1164 genes were down-regulated, which were involved in primary metabolic functions, including carbon and amino acid metabolism. Interestingly, these were not spatially structured across the genome. This work has provided some initial insight into the genetics of biofilm formation.

Evaluation of differential gene expression in *A. niger* biofilms formed on polyester cloth was performed. It was shown that genes encoding some lignocellulolytic enzymes and some regulatory genes showed that *eng1*, *eglA*, *eglB*, *eglC*, *exo* and *xynB* genes (coding for endoglucanases, a cellobiohydrolase and a xylanase respectively) are differentially expressed in biofilm fermentation. Likewise, the regulatory genes *xlnR* (cellulase activator) and *creA* (cellulase repressor) showed time-related expression patterns, indicating that a different regulatory system may act in biofilms (Villena *et al.*, 2009a). The intracellular proteome of *A. niger* biofilms was recently compared with that of the conventionally grown free-living submerged cultures. In biofilm cultures, 19% and 32% of the selected protein spots were over-expressed and differentially expressed, respectively, compared to 44% and 7%, respectively, in free-living cultures (Villena *et al.*, 2009b). It was demonstrated that *A. niger* biofilms differentially expressed a putative calcium P-type ATPase, which is important both in the homoeostatic maintenance of calcium concentration in the endoplasmic reticulum, and in cation-dependant functions of Golgi apparatus (Vashist *et al.*, 2002); this protein is probably involved in cAMP-mediated signalling (Bencina *et al.*, 2005).

Biofilm matrix

Biofilms require the production of an EPS to satisfy the basic definition, which provides protection from hostile factors, such as host immunity and antifungal agents (Ramage *et al.*, 2009). The presence of extracellular hydrophobic matrix composed of galactomannan, alpha-1,3 glucans, monosaccharides, polyols, melanin and proteins including major antigens and hydrophobins in an aerial static *A. fumigatus* biofilm model was recently demonstrated (Beauvais *et al.*, 2007). This model was developed to study the characteristics of a fungus ball, opposed to using the typical submerged shaking culture system. Within the ball, hyphae are agglutinated and collectively form a macrocolony of highly branched hyphal elements that are tightly associated with one another. This study demonstrated that hydrophobic matrix cohesively bound hyphae together, and that the matrix increased with maturity of the developing structure. Further studies reported that a new galactosaminogalactan and the galactomannan were the major polysaccharides of the *in vivo A. fumigatus* EPS (Loussert *et al.*, 2010). For *A. niger*, after germination upon a support, the new hyphae also produce an EPS (Villena & Gutierrez-Correa, 2007b). Singhal *et al.* (2011) recently reported that primary epithelial cells could support the growth of biofilms under flow conditions that were also associated with significant EPS production compared with biofilms formed under static condition (Singhal *et al.*, 2011). The production of EPS has also been reported elsewhere, where it is shown to be produced on polystyrene and on CF bronchial epithelial cells (Seidler *et al.*, 2008). This study also reported that biofilm cells attaching to epithelial cells exhibited decreased sensitivity to antifungal drugs. Whilst the precise role of the EPS is not known, it is hypothesized that it plays a significant role in antifungal resistance by preventing diffusion. This is supported from data emerging from the *C. albicans* biofilm field, where it was demonstrated that EPS expression (specifically beta-glucans), encoded through *fks1*, sequesters antifungal agents and reduces susceptibility (Nett *et al.*, 2010a). Figure 2 illustrates the presence of EPS within *A. fumigatus* biofilms.

Biofilm resistance

Antifungal resistance is a defining characteristic of fungal biofilms. In *A. fumigatus*, biofilms antifungal resistance has been reported (Beauvais *et al.*, 2007; Mowat *et al.*,

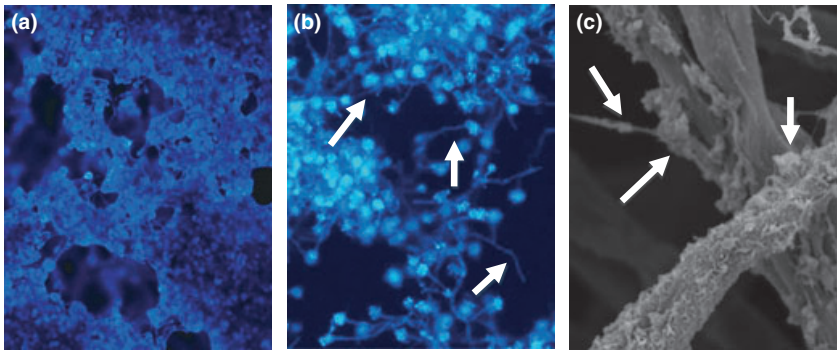

Fig. 2. *Aspergillus fumigatus* biofilms are encased in EPS. EPS is produced by *A. fumigatus* biofilms during maturation. (a) Calcofluor white fluorescently stained 24 h biofilm demonstrate a dense matrix of cellular material, which when (b) treated with EDTA removes the matrix to show the individual hyphae within the biofilm (denoted by arrows). (c) A scanning electron micrograph illustrates the presence of polymeric material attached to hyphae (denoted by arrows).

2007; Seidler *et al.*, 2008; Fiori *et al.*, 2011), which has been shown to be phase dependant (Mowat *et al.*, 2008b). Here, three phases of biofilm growth (8, 12 and 24 h) were investigated to assess the effects of antifungal agents on different phases of biofilm. Clear differences in susceptibility were observed in each biofilm population, where younger biofilms (8 h) were significantly more susceptible than intermediate (12 h) and mature biofilms (24 h) (Mowat *et al.*, 2008b). Our recent study, supports the concept that this phase resistance is correlated with efflux pump activity. This study reported that efflux activity increases with biofilm maturity, and that sensitivity to voriconazole could be improved through the use of a competitive inhibitor. Transcriptomic analysis showed that maximum activity associated with the early filamentous phase (12 h), and in defined clinical isolates, maximal expression of *mdr4* correlated with the highest increase in resistance in 12 h biofilm populations. Conversely, expression of this gene was minimal at 24 h, suggesting phase dependant efflux activity (Rajendran *et al.*, 2011). It was therefore speculated that efflux pump activity plays a contributory role to antifungal resistance. It is conceivable that *A. fumigatus* resistance within biofilms is tightly regulated and biphasic, involving efflux pumps in the early to intermediate phases of biofilm development, with EPS material being produced towards maturity (Nett *et al.*, 2010a; Rajendran *et al.*, 2011).

Mixed biofilms

Clinically, moulds have become increasingly recognized in the CF lung, however, their definitive role is yet to be established and fully understood (Pihet *et al.*, 2009). Further clinical relevance for the role of the *A. fumigatus* biofilm phenotype and the role of filamentation are provided from our knowledge of the CF lung microbiome (Burns *et al.*, 1998; Cimon *et al.*, 2001; Bakare *et al.*, 2003). *Aspergillus fumigatus* is commonly isolated from here; however, the levels of disease are relatively low suggesting some interactive behaviour. Our recent *in vitro* study, aimed to investigate how *A. fumigatus* interacts with *Pseudomonas aeruginosa*, the primary CF biofilm pathogen (Mowat *et al.*, 2010). *Aspergillus fumigatus* biofilm formation was shown to be inhibited by direct contact with *P. aeruginosa*, but preformed biofilms were unaffected. A secreted heat-stable soluble factor was also shown to exhibit biofilm inhibition. Subsequently, co-culture of *P. aeruginosa* quorum sensing (QS) mutants (*ΔlasI* and *ΔlasR*) did not significantly inhibit *A. fumigatus* biofilms and filamentation to the same extent as that of the PA01 wild type, both by direct and by indirect interaction. It was hypothesized that these were related to QS molecules and demonstrated that sessile cells could be inhibited and disrupted in a concentration-dependent manner by short carbon chain molecules (decanol, decanoic acid and dodecanol) analogous to QS molecules. Overall, this suggests that small diffusible and heat-stable molecules may be responsible for the competitive inhibition of filamentous fungal growth in polymicrobial environments such as the CF lung, and this could be harnessed as a potential therapeutic strategy. This is particularly important, given the high levels of biofilm resistance to common chemotherapeutic agents (Mowat *et al.*, 2008b; Seidler *et al.*, 2008; Nett *et al.*, 2010a; Fiori *et al.*, 2011), which is often associated with biofilm specific phenotypes such as EPS production.

Concluding remarks

In this article, we have briefly discussed morphological, physiological and molecular aspects of both clinically and industrially important *Aspergillus* biofilms, and have

shown where and why they are important. Clinically, it is clear that much can be learned from the industrial platforms described herein. *Aspergillus fumigatus* biofilms are a problematic clinical entity, and given their recalcitrance to antifungal agents, understanding the molecular pathways that define this clinical resistance is an important step towards identifying new therapeutic targets.

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